THE KANSAS UNIVERSITY SCIENCE BULLETIN.

Vol. IX, No. 10—December, 1914.

(Whole Series, Vol. XIX, No. 10.)

CONTENTS:

A STUDY OF THE DIMENSIONS OF THE CHROMOSOMES OF THE SOMATIC CELLS OF AMBYSTOMA, ... *Jas. B. Mack.*

PUBLISHED BY THE UNIVERSITY,

LAWRENCE, KAN.

Entered at the post-office in Lawrence as second-class matter.

5 2496

KANSAS STATE PRINTING OFFICE.
W. C. AUSTIN, State Printer.
TOPEKA. 1915.

THE KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. IX, No. 10.] DECEMBER, 1914. [WHOLE SERIES VOL. XIX, No. 10

A Study of the Dimensions of the Chromosomes of the Somatic Cells of Ambystoma.

JAS. B. MACK.

Contribution from the Zoölogical Laboratory No. 212.

[Submitted as a partial fulfillment of the requirements for the degree of Master of Arts.]

FOR several years past cytologists have devoted much time to the study of the chromosomes of the germ cells, the most thorough work having been done upon the Insecta, while some has been done upon the Amphibia. But to my knowledge scarcely any investigation has been attempted upon the chromosomes of somatic cells.

It is the purpose of this paper to present some results obtained from the study of the somatic cells of Ambystoma in an attempt to discover the relation between the chromosomes of cells of the derivatives of the mesoblast and those of the ectoblast. The work along this line was suggested to the author by Dr. C. E. McClung and we have hoped that we might obtain some data which might aid us toward the establishment of a correlation of individual chromosomes and definite somatic characters.

Since the work was begun, however, a paper has appeared by Capt. C. F. U. Meek on "A Metrical Analysis of Chromosome Complexes" in which he draws the conclusion that chromosomes of different phyla possess diameters of constant size, and all those above Nemathelminthia have a diameter of .83μ, and that the somatic chromosomes are identical with those of the germ cells. This has led me to conduct my investigations along a somewhat different line from that which I originally intended in order to

corroborate his statements if possible. To make the arguments still more conclusive, in addition to the work upon Ambystoma, I made a few measurements and drawings of the chromosomes of Gryllus. If Meek's theory can be established as a law which will hold throughout the animal kingdom it will be of inestimable value.

MATERIAL AND METHODS.

The material for the study of both *Ambystoma* and *Gryllus domesticus* was prepared in the laboratories of Kansas University. The Ambystoma was fixed in Flemming's Fluid and stained with Heidenhain's iron-hæmatoxylin. The slides for the Gryllus were kindly loaned me by Prof. W. J. Baumgartner. These were all fixed in Flemming's fluid; some were stained with iron-hæmatoxylin and some with Flemming's triple stain.

The preparations were studied by means of a Leitz apochromatic oil immersion $\frac{1}{12}$ a objective and a No. 4 ocular and a No. 18 compensating eye piece. The source of illumination for most of the work, and especially for the measurements and drawings was ordinary daylight projected upon the mirror of the microscope by means of a funnel made of heavy black paper inserted in other heavy paper which covered the greater part of the window, thus admitting only a small amount of light between the shade and the heavy paper. Part of the work was done by gas light with a Welsbach burner, the rays passing through a copper sulphate solution.

The drawing board was built on a level with the microscope stage. The measurements and drawings were made by the aid of an Abbe camera lucida, so adjusted that the center of each measurement and each drawing was 100 mm. from the center of the objective. The measurements were all made with the draw tube adjusted so that the distance from the drawing table to the upper part of the ocular was 231 mm., thus giving a magnification of 4000 diameters. The drawings were made with the draw tube adjusted so that the distance was 211 mm., giving a magnification of 3200 diameters. The drawings represented in the plate here are reproduced at 2145 diameters. The magnifications were determined by means of a Zeiss stage micrometer.

OBSERVATIONS.

It will not be necessary to go into details concerning the early prophase of mitosis as that has already been dealt with by other investigators. By a count of the chromatin knots and the chromosomes of the telophase, and especially of the metaphase, I find

the number to be 24 in the cells of the various tissues, agreeing with the number in the spermatogonial and primary spermatocyte as given by the *Schreiners and Meek. Baumgartner seemed to discover a difference in the form of the chromosomes; Sutton a difference in size; and Meek a difference in measurements. I wish to apply these tests to the somatic cells to determine whether by a study of the form, size and dimensions we can arrive at some definite conclusions concerning their relation to the germ cells.

I made a careful study of several hundred cells, and made measurements of the chromosomes of most of them, and compared those of the neuroplasm with those of the ectoplasm, the muscle, the connective tissue and the pigment tissue. In all cases I found them grouped about the equatorial plate in an irregular manner. They are always in the U or V shape with the spindle fibres attached one at the vertex of each chromosome. These fibres are almost invariably attached in such a way that the two arms of the V are of unequal length.

As will be seen in figs. 4 and 5 the chromosomes in the same cell are often irregular in outline, thick in some places and thin in others. The chromosomes of the connective and muscular tissue, while resembling each other in form and size, have some differences and are not like those of other tissues. Those of the muscular tissue are rough and short, while those of the connective tissue are short, thick and smooth. Those of the pigment cells are always very smooth and also much longer in proportion to their breadth and can invariably be distinguished by their high staining qualities, always being a bright violet in the preparations studied.

Some of the chromosomes differ in size because of difference in tissue and also because of the difference in the age of the tissue, as will be brought out more definitely a little later. They also differ in size in individual cells but the difference is so slight in any one cell that it is difficult to determine without accurate measurements, neither is it possible to arrange them in any such system as some authors have done, of three small, two medium and five long ones, etc. Without a doubt they have an individuality and "these various shapes are an expression of the individual characteristics of the various chromosomes" as †Baum

* A. and K. E. Schreiner. Neue Studien uber die Chromatinreifung der Geschlechtszellen.

†Baumgartner ('04). Some New Evidence for the Individuality of the Chromosomes.

gartner ('04) has stated. But there is not sufficient difference either in form or size to substantiate a theory of persistence of this individuality.

In studying the size of the chromosomes more carefully I made sketches of from five to eighteen chromosomes in each of fifty cells in the Neuroplasm, 21 cells in the ectoplasm, 15 cells in the muscle, 15 cells in the connective and 10 cells in the pigment. I did this for each of the three different ages of the Ambystoma, using the camera lucida and micrometer, as described previously. The accompanying table gives a record of the results obtained. For convenience we will use the series number of the slide 628 to represent the youngest and 630 the oldest.

No. of slide.	No. cells.	Tissue.	Av. Width.	Metaphase.	Anaphase.	Extremes.
			μ	μ	μ	μ μ
628	50	Nerve	.87	.85	.77	.65 to 1.02
	21	Ectoplasm	.83	.82	.77	.55 to 1.10
	15	Muscle	.663	.67	.67	.63 to .83
	10	Pigment	.725	.715	.70	.70 to .80
	15	Connective	.767	.73	.752	.65 to .98
629	50	Nerve	.797	.83	.71	.60 to 1.05
	21	Ectoplasm	.775	.79	.73	.63 to 1.20
	15	Muscle	.80	.78	.50	.50 to 1.08
	10	Pigment	.85	.75	.73	.65 to 1.30
	15	Connective	.735	.80	.65	.55 to 1.20
630	50	Nerve	.75	.80	.65	.53 to 1.70
	21	Ectoplasm	.75	.75	.68	.57 to 1.10
	15	Muscle	.70	.68	.53	.48 to .93
	10	Pigment	.70	.68	.73	.53 to .92
	15	Connective	.65	.73	.70	.58 to .97

Great care was taken to use only chromosomes in which the complex lay perpendicular to the line of sight lest there be a possibility of foreshortening. In many cases it was possible to find a complex, or nearly a complete complex, in which the diameter was uniform, and in which the length seemed to form some kind of a mathematical series if not an arithmetical one. But this was the exception rather than the rule. We know that the larger the number entering into an average, the less is the liability of error, and the more general and reliable becomes our deduction. For this reason I tested 50 nerve cells instead of one or two. I measured the diameter of from 5 to 18 chromosomes, in each of these cells and worked out the average for each cell and then the average for the entire 50 nerve cells. We find by referring to the table here for No. 628 that the average width is .87 μ. The average for the metaphase and anaphase alone are .85 μ and .77 μ respectively. But in the individual cells there is a greater difference. The averages for the cells range from .65 to

1.02 μ. If we compare these figures with the corresponding results recorded for the nerve tissue of Nos. 629 and 630 we notice a marked difference in the diameter, which seems to decrease as the age increases. This holds true with those of the different phases as well. The variation in the averages continues very great. In No. 630 it ranges from .53 μ to 1.70 μ. The cells showing mitosis are far more numerous in No. 628 than in the others and the amount of nerve tissue is greater also as might be expected. The results for the other tissues were worked out in a similar manner. The number of cells showing mitosis in the other tissues is much less than in the nerve tissue, so that 21, 15 and 10 were about all the good specimens that could be found in these different tissues.

In the ectoplasm for No. 628 the general average is .83 μ, for the metaphase alone it is .82 μ with various averages for individual cells ranging from .55 μ to 1.10 μ. If we compare these figures with those for the chromosomes of the ectoplasm of No. 629 and No. 630 we find in one case the average is .775 μ and in the other .75 μ and for the metaphase alone it is .79 μ and .75 μ, with still greater extremes in the general averages ranging from .63 μ to 1.20 μ in one case and .57 μ to 1.10 μ in the other. As in the nerve plasm the diameter decreases as age increases.

In the muscle the average for No. 628 is .663 μ and for the metaphase .67 μ with a variation in extremes of only .63 μ to 83 μ. Contrary to the action of the chromosomes in the nerve plasm and ectoplasm, the general average of diameters increases in No. 629 to .80 μ and .78 μ for metaphase and then decreases slightly in No. 630 to .70μ for the general average and .68 μ for the metaphase, with great variations and extremes.

The chromosomes of the pigment cells have an average diameter in No. 628 of .725 μ. The metaphase has a diameter of .715 μ. The chromosomes in No. 629 of the same tissue has a diameter of .85 μ, the metaphase of .75 μ. The extremes are .65 μ and 1.30 μ. The diameters in the pigment cells seems to be greatest in No. 629 and least in No. 630 both for the metaphase and for the general average, neither following the results for the nerve nor for the muscle.

Comparing the results of the pigment with those of the nerve-plasm, ectoplasm and muscle we find that the diameter in No. 628 is greater than the diameter for the muscle and less than for the nerveplasm and ectoplasm. In No. 629 it is greater than in any of the others and in No. 630 it is less than any of the others

except for the muscle, which it equals. Considering the metaphase alone the average is less than any of the other metaphases for No. 628, the same in No. 629 and the same in No. 630.

Considering the chromosomes of the connective tissues we find the average diameter for No. 628 to be .767 μ, for the metaphase alone it is .73 μ with the greatest diameter found in the anaphase. In No. 629 the average is .735 μ and the greatest diameter is found in the metaphase. The extremes of the averages being .55 μ and 1.20 μ. In No. 630 it is .65 μ and for the metaphase it is .73 μ, this being again above the anaphase. Here again we find the greatest diameter in the younger, and decreasing with age, but for the metaphase alone it is greatest for No. 629.

Comparing those of the connective tissue with those of the other tissue we find the total average less than for any of the others in Nos. 629 and 630 but greater than those of the muscle or pigment in No. 628.

It is quite conclusive from these figures that there is a difference in chromosome diameters due to age, and that there is a difference in different tissues of the same age, but this variation is not constant. The diameter is neither constant in all phases of the same tissue, as Meek declares, nor is it the same for any one phase in all the tissues. The diameter is not necessarily constant for all the chromosomes in any one somatic cell of any particular tissue, although it is possible this may occasionally happen. The diameters of the chromosome of the neuroplasm and the ectoplasm decrease as the age increases.

It was practically impossible to find cells where all the chromosomes of a complex lay in a plane perpendicular to the line of vision so that one could make accurate measurements of the lengths and diameters of all the chromosomes of a complex, as will be seen by noticing in Figs. 2, 3, 4, and 5 the various positions of the individual chromosomes. The dimensions of 25 chromosomes were taken and the volumes worked out to see if any constancy existed there. The ends were considered to be rounded. The two ends making a sphere whose diameter equalled the diameter of the chromosome and the intermediate space being a cylinder, whose diameter is the diameter of the rod and the length equal to the length of the chromosomes, less its diameter. The volumes ranged from 1.54 cu. μ to 17.94 cu. μ. In a single cell the lengths ranged from 7.5 μ to 10 μ, and the diameter from .63 μ to 1.00 μ and the volume from 2.84 μ to 5.63 cu. μ.

*Meek ('12) declares that the diameters of the chromosomes

* C. F. U. Meek. A Metrical Analysis of Chromosome Complexes.

for this phyla are constant and equal to .83 μ and on page 12 states that "a study of the lengths of the ordinary chromosome rod leads to the discovery that they constitute a series in arithmetical progression the short chromosomes being consecutive members." On page 25 he asserts that "the complexes of a species and its variety appear to be identical; differences, if existing, are too small to be recognized"; also that "the somatic chromosomes are identical with those of the germ cells." He further adds that "the total volume of ordinary chromosomes is the same in the spermatogonial and primary spermatocyte metaphases, whereas only half this amount appears in that of the secondary spermatocyte." If the somatic chromosomes are identical with those of the germ cells we should naturally expect them to follow his theory concerning the spermatogonial and the first spermatocyte chromosomes in diameter, length, and volumes. From a careful study of my results previously recorded it is very evident that in no case was I able to find such a constant diameter nor such a regularity in the variation in length of the chromosomes in the material at hand.

In order to ascertain whether this variation in size was peculiar to the somatic cells only, I measured from seven to ten chromosomes in the metaphase in each of twenty-two cells of the first spermatocyte in *Gryllus domesticus*, and those of one in the anaphase, and those of two in the metaphase of the primary spermatogonian, the lengths of the chromosomes in one of the best cells of the spermatocyte were 3.5, 3.75, 2.5, 2.25, 2.25, 2.25, 2, 2, 5, and 1.5 μ. The corresponding diameters were 1.25, 1.75, 1.5, 1.75, 1, 1, 1, 1, 1.25, and 1 μ. The lengths of another were 3, 2.5, 2.25, 2.25, 2.25, 2, 2, 1.5, 1.5, and 1.25 μ, and the corresponding diameters were 1.25, 1.5, 1.5, 1.25, 1.25, 1.75, 1.5, 1.25, 1, and 1.25 μ. The lengths for one of the best spermatogonial cells were 3, 2.5, 2.5, 2.5, 2.25, 2.25, 2, and 2 μ, while the diameters were .50, .75, .75, .62, .75, .75, 1.25, and .50 μ. I could in none of these cases, as can easily be seen, find an absolute constant diameter as Meek mentions for the same animal in the same species, although there were several diameters which were identical. Nor could I find the relation between the lengths which could be called "an arithmetical series whose common difference was less than half of the diameter." In order to avoid any error which might occur, due to fixation, I was careful to choose cells which were similarly located in the testis.

In order that I might discover whether I was in error, I next chose two cells, one of the primary spermatogonial and one of the first spermatocyte as illustrated in Baumgartner's paper on *Gryllus domesticus*. In the spermatogonia the diameter was .514 μ, but not .83 μ, as Meek gives for the same material, and the lengths range from .86 to 2.41 μ, and not from .8 to 6.71 μ. In the first spermatocyte the diameter of seven was .514 μ, that of one was .69 μ and that of another was 1.04 μ, excluding the accessory; the lengths ranging from 1.04 to 4.13 μ.

SUMMARY.

The number of chromosomes is twenty-four in the somatic cells of the Ambystoma, agreeing with the number in the primary spermatocyte.

The chromosomes are grouped about the equatorial plate in a U or V shape with the spindle fibres attached, one at the vertex of each chromosome, so that the arms of the V are of unequal length.

They differ in size and form in different tissues, being thick and rough in outline in some, and long, slender and smooth in others.

The chromosomes of the pigment cells are long and thin in proportion to their length and possess higher staining qualities than the others.

It is impossible to arrange the chromosomes in any one cell in any systematic classification according to form or size.

There is a difference in chromosome diameter due to age and there is a difference in different tissues of the same age, but this variation is not constant.

The diameter is not constant in all phases of the same tissue.

The diameter is not constant for all the chromosomes in any one somatic cell of any particular tissue.

The diameters of the chromosomes in neuroplasm and ectoplasm decrease as the age of the animal increases.

The diameter and volume of the phyla are not constant, neither do their lengths constitute an arithmetical series.

The volumes range from 1.54 to 17.94 cu.μ and in a single cell the volume ranged from 2.84 to 5.63 cu.μ.

The germ cells may have several diameters which are identical but they do not posses an absolutely constant diameter, nor do the lengths form an arithmetical series.

I wish to express my appreciation to Dr. C. E. McClung for starting me on this material and to Prof. W. J. Baumgartner for suggestions and for the kindly interest shown in the work. I am also indebted to Dr. T. C. Frye for permitting me to use a room in the Puget Sound Marine Station.

Since this paper has been sent to the publisher new papers have appeared by Meek on "A Further Study of the Metotic Spindle in the Spermatocytes of Forficula auricularia," in which he says that "new evidence before us shows us that the previous proposition put forward can not be universal," and that, "so far cytometrical investigations seem to have yielded only negative generalizations," thus agreeing with our results.